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PARAOXONASE 1 GENE POLYMORPHISM AND ITS INFLUENCE ON CARDIOVASCULAR THREAT IN TYPE 2 DIABETES MELLITUS

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ABBREVIATION

Sr No	Abbreviation	Full form
1.	ADA	American Diabetes Association
2.	CVD	Cardiovascular disease
3.	DM	Diabetes Mellitus
4.	T1DM	Type 1 Diabetes Mellitus
5.	T2DM	Type 2 Diabetes Mellitus
6.	IDF	International Diabetes Federation
7.	ROS	Reactive oxygen species
8.	NO	Nitrogen Oxide
9.	LDL	Low Density Lipoprotein
10.	HDL	High Density Lipoprotein
11.	VLDL	Very Low Density Lipoprotein
12.	LCAT	Lecithin Cholesterol Acyltransferase
13.	SNP	Single Nucleotide Polymorphism
14.	TG	Triglycerides
15.	4-HDOHE	4-Hydroxy-Docosahexaenoic
16.	5-HETEL	5-Hydroxyeicosatetraenoic Lactone
17.	PON 1	Paraoxonase 1
18.	WHO	World Health Organisation
19.	NIDDM	Noninsulin-dependent Diabetes Mellitus
20.	IDDM	Insulin-dependent Diabetes Mellitus
21.	MRDM	Malnutrition-related Diabetes Mellitus
22.	FBG	Fasting Blood Glucose
23.	EDTA	Ethylene Diamine Tetra Acetic Acid
24.	DNA	Deoxyribonucleic acid

25.	PCR	Polymerase Chain Reaction
26.	SEC	Seconds
27.	OPD	Out Patient Department
28.	HbA1c	Glycatedheamoglobin
29.	OGTT	Oral Glucose Tolerance Test
30.	NGSP	National Glycohemoglobin Standardisation Programme
31.	mRNA	messenger RNA
32.	RFLP	Restriction fragment length polymorphism
33.	FPG	Fasting Plasma Glucose
34.	IGT	Impaired Glucose Tolerance
35.	IFG	Impaired Fasting Glucose
36.	MCP-1	Monocyte Chemoattractant Protein-1
37.	RAAS	Rrenin-Angiotensin-Aldosterone System
38.	OP	Organophosphate

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1. INTRODUCTION

Long seen as a condition with little bearing on global health, diabetes mellitus (DM) is suddenly emerging as one of the major public health concerns of this 21st century (Zimmet, et al. 2001). It ranks fourth or fifth in developed countries as the primary cause of death and is the most prevalent non-communicable disease globally (Roglic, et al. 2010). There has been a sharp rise in the global diagnosis rate of DM during the last 20 years. Type 2 diabetes is the specific cause of this DM epidemic, which is occurring in both industrialised and developing nations (IDF Diabetes Atlas, 2012). Most individuals with type 2 diabetes and two-thirds of those with poor glucose tolerance have insulin resistance (Bonora et al., 1988) and the risk of cardiovascular diseases (CVD) is considerably higher in both of these categories (Saydah et al., 2001). Patients with diabetes mellitus have an outstanding expanded hazard of dreariness and passing from CVD. According to (Johnstone MT et al 2007), the cardiovascular metabolic syndrome includes factors that accelerate the atherosclerotic process in diabetes, including hyperglycemia, insulin resistance, atherogenic dyslipidemia, coagulation abnormalities, platelet and endothelial dysfunction, and oxidative stress. Reactive oxygen species generation overwhelms the antioxidant systems' ability to protect against oxidative stress, which is a common feature of diabetes problems (Jones, et al. 2006). According to Jain et al. (2006), hyperglycemia causes elevated oxidative stress as well as dysfunction in monocytes and endothelial cells. Reactive oxygen species (ROS) have been implicated in the pathophysiology of cardiovascular illnesses. Numerous signalling molecules and cellular pathways, including transcription factors, protein tyrosine phosphatase, protein tyrosine kinase, mitogen activated protein kinase, calcium transporting system, and protein modification, are targeted by ROS generated in vascular walls and vascular smooth muscle. By decreasing the bioavailability of Nitric Oxide (NO), preventing endothelium-dependent vasodilatation and endothelial cell growth, promoting endothelial cell migration, activating adhesion molecules and inflammatory

response, and causing endothelial dysfunction—the first stage leading to hypertension and atherosclerosis—ROS contribute to vascular dysfunction and remodelling through oxidative damage (Yung et al., 2006). Low levels of HDL cholesterol, elevated levels of lipoproteins rich in triglycerides, particularly VLDL, and a preponderance of proatherogenic small dense low density lipoprotein (LDL) are all related with dyslipidemia, which is a condition associated with type 2 diabetes. Prolonged low-grade inflammation and oxidative stress are closely linked to this lipid profile. Diabetes type 2 may affect the amounts and functions of HDL-associated enzymes, such as lecithin cholesterol acyltransferase (LCAT), platelet activating factor acetylhydrolase (PAF-AH), and paraoxonase 1 (PON1) (Kontush and Chapman, 2008).

In order to effectively manage patients with type 2 diabetes, it is imperative to track changes in cardiovascular problems using PON1. When compared to serum PON1 activities in healthy control subjects, it was discovered that diabetic patients had considerably lower serum PON1 activities (arylesterase and paraoxonase) (Juretic et al., 2006; Rosenblat et al., 2008). Likewise there is growing evidence that PON1 and DM are related (Hofer et al., 2006). PON1 and HDL are physically linked together. According to studies by (van Wijk et al. 2006) and (Saeed et al. 2007), people with type 2 diabetes may exhibit low HDL levels (Sarkar et al., 2006) or levels that are equal to those of healthy individuals. However, compared to those in normoglycemic people, the HDL apoproteins in diabetics may be substantially glycated (Norata et al, 2004).

Protein PON1 has an atomic mass of 43 kda and comprises of 354 amino acids (Primo-Parma et al., 1996). Human PON1 is only found in serum that contains HDLs and is produced in the liver and secreted into the blood (Mackness MI, et al. 1989). According to Rosenblat et al. (2006), the active site of PON1 has been demonstrated to mediate two main anti-atherogenic functions: protecting LDL from oxidation and stimulating HDL-mediated macrophage cholesterol efflux. It is still unknown how PON1 functions physiologically. PON1's arylesterase and paraoxonase activities have been shown using xenobiotic substances, which

are not the enzyme's normal substrates. Nevertheless, data supporting PON1's lactonase function has started to surface in the literature in recent years (Draganov et al., 2004; Teiber et al., 2003). The ancestral enzyme activity is lactonase. PONs have been demonstrated to act on a wide range of physiologically significant lactones, such as statin medications, homocysteine thiolactone, homoserine lactones, and derivatives of oxidised fatty acids (Harel et al., 2004; Teiber et al., 2004). PONs' classification as lactonases broadens the pool of possible physiological substrates with particular applications. Participation in lipid peroxidation continues to play a significant role. This process is driven by 4-hydroxy-docosahexaenoic corrosive (4-HDOHE) and 5-hydroxyeicosatetraenoic lactone (5-HETEL), which are basically related to lipoxins and are items of both enzymatic and non-enzymatic oxidation of arachidonic corrosive and docosahexaenoic corrosive, individually (Gaidukov et al., 2005). According to (Costa et al. 2003a), oxidised LDL (ox-LDL) and lipoxins both have strong inflammatory properties. They may also serve as the substrate for PON1's connection to atherosclerosis, an inflammatory illness. Additionally, PON1's homocysteine thiolactonase activities contribute to the involvement of PON1 in oxidative stress and vascular disease (Costa et al., 2003b; Draganov et al., 2000).

Numerous studies demonstrated that the paraoxonase 1 (PON1) phenotype, which reflects the activity of the enzyme, is a stronger predictor of vascular disease than individual genetic variants in the PON1 gene. It suggests that variations in PON1 activity may influence cardiovascular risk (Jarvik GP, et al. 2000). Li Z et al investigated the genetic determinants of T2DM in a Chinese population that highlights the role of genetic variations in the PON1 gene in predisposing individuals to T2DM associated with cardiovascular complications (Li Z, et al. 2020). Kamboh et al explored the genetic associations between apolipoprotein polymorphisms, including PON1, and serum lipoprotein lipid levels, which are key risk factors for cardiovascular disease, in African black populations (Kamboh MI, et al. 1999). Mackness B et

al investigated the association between serum paraoxonase activity and the risk of coronary events in a general population cohort. It provides evidence that low paraoxonase activity is a predictor of coronary events, suggesting its potential role in cardiovascular risk assessment (Mackness B, et al. 2003).

In the present, study we are trying to establish an association between PON1 gene and its association with type 2 diabetes mellitus that might increase the risk of cardiovascular diseases.

2. AIMS AND OBJECTIVES

Aim: To study Paraoxonase 1 gene polymorphism and its influence on cardiovascular threat in type 2 diabetes mellitus.

Objectives:

- To find the association between the identified SNPs on PON1 gene and the risk of CVD in T2DM patients.
- To determine the hyper glycaemia and Lipid profile levels.

Research question:

Does Polymorphism in SNPs in Paraoxonase 1 gene contribute to the development of Type 2 diabetes mellitus and cardiovascular risk?

Hypothesis:

Polymorphism in the PON1 gene will have a direct and significant relationship and influence the cardiovascular threat in type 2 diabetes mellitus.

Null hypothesis:

Polymorphism in the PON1 gene will not have a direct and significant relationship on influencing the threat of cardiovascular threat in type 2 diabetes mellitus.

3. REVIEW FOF LITERATURE

3.1. Diabetes mellitus

3.1.1. General concept

Diabetes mellitus (DM) includes several common metabolic disorders characterized by high blood sugar levels. There are various types of DM caused by a combination of genetic, environmental, and lifestyle factors. The factors contributing to high blood sugar levels in DM can include reduced insulin secretion, decreased glucose utilization, and increased glucose production (Longo DL, et al. 2012). The World Health Organization's (WHO) Expert Committee on Diabetes defined diabetes in 1980 as a state of persistent hyperglycemia that can be caused by a variety of hereditary and environmental variables that frequently work in concert. Defects in insulin secretion, action, or both can cause hyperglycemia. Disturbances in the metabolism of fat, protein, and carbohydrates result from this imbalance (Galicia-Garcia, et al. 2020). The main consequences of diabetes mellitus are long-term harm, organ failure, and malfunction. The following are typical symptoms of diabetes mellitus: thirst, polyuria, polydipsia, blurred eyesight, weight loss, and infections. In its most extreme forms, ketoacidosis, also known as a nonketotic hyperosmolar state, can occur. This condition can cause stupor, coma, and, in the event that treatment is not received, death. The majority of the time, symptoms are either mild or nonexistent; as a result, hyperglycemia severe enough to induce pathological and functional abnormalities may exist for a considerable amount of time before a diagnosis is obtained. Despite the wide range of clinical symptoms and other contributing factors, hyperglycemia was thought to be the most significant risk factor for the pathophysiology of diabetes. Evidence has mounted over the last 30 years indicating that various etiological mechanisms (genetic, environmental, or immunologic processes) are

significant contributors to the pathogenesis, clinical course, and emergence of complications associated with the "diabetic state" (Dong H, et al 2024).

3.1.2. Genetic Factors of Diabetes

The pathogenesis of type 2 diabetes is controversial, but it is generally accepted that the disease has strong genetic and environmental (acquired) components (Stančáková A et al, 2016); 1) Its inheritance is polygenic (DeForest N, et al. 2022), which means that the disease must simultaneously develop due to the presence of multiple abnormal genes or polymorphisms; 2) Impairment of insulin sensitivity and insulin secretion, both of which are genetically controlled (Batista TM, et al. 2021), and both of which are significant elements in its pathogenesis; 3) The majority of patients are obese; and 4) most patients are obese (Chen J, et al. 2023). The fact that a wide range of environmental and genetic factors, including age, gender, ethnicity, physical fitness, food, smoking, obesity, and fat distribution, affect insulin sensitivity and secretion poses a significant challenge to our understanding of the genetic foundation of type 2 diabetes. It is crucial to stress that while many of these might be influenced by genetics (Tan Q, et al. 2023), the genes might not always correspond to particular diabetes genes. Although most insulin-resistant fat persons do not develop type 2 diabetes, one may argue that the insulin resistance discovered in DM was genetic (Scott RA, et al., 2014). However, this would not reflect a particular diabetes gene. However, since the majority of people with a serious enough genetic abnormality would acquire diabetes and the majority of people without diabetes would not have this gene, a mutation in the insulin receptor gene that causes insulin resistance might be classified as a diabetes-specific gene. Therefore, it's critical to distinguish between genes that cause diabetes and genes that are associated to the disease (such as those that control hunger, energy expenditure, and the buildup of intra-abdominal fat) (Bomba L, et al. 2022).

3.1.3 Cardiovascular in T2DM

Cardiovascular disease and diabetes mellitus are closely related (Szuszkiewicz-Garcia MM, et al. 2014). People with diabetes have a higher prevalence of cardiovascular disease than people without the disease (Wang X, et al. 2023). Even before fasting plasma glucose levels are high enough to be diagnosed as diabetes, this risk gradually rises with rising levels (Yang CP, et al. 2020). One of the main causes of death for people with DM is CVD (Goff Jr DC, et al. 2021). People with type 2 diabetes mellitus have a two-fold greater risk of cardiovascular mortality compared to healthy people, and CVD accounts for almost half of the deaths of people with DM (Rawshani A, et al. 2018). Thus, early identification and treatment of CV hazards in diabetic patients is one of the main objectives of diabetes management (Care D, 2022). Comprehending the mechanisms underlying the trait-disease or disease-disease link is crucial in order to avert severe consequences (Zhang P, et al. 2024). The intricate pathophysiologic process underlying the relationship between type 2 diabetes and cardiovascular disease is a challenge for managing cardio metabolic risk factors in patients with T2DM (Yadav U, et al. 2023). The risk of cardiovascular disease is exacerbated by traditional cardio metabolic risk factors, including obesity, dyslipidemia, and hypertension, all of which are prevalent in type 2 diabetes (Aguilar-Ballester M, et al. 2021). A number of other characteristics that are frequently seen in individuals with type 2 diabetes have also been shown in numerous research. These factors include an increased risk of insulin resistance, a hereditary predisposition, and hypoglycemia during therapy (Tsimihodimos V, et al. 2024). These elements are thought to represent a significant obstacle to CVD prevention (Lee CL, et al. 2024). However, there are several practical methods for T2DM patients to avoid CVD (Pagidipati NJ, et al. 2023). For example, a wealth of evidence suggests that modifying one's lifestyle might both successfully improve one's risk of CVD and avoid CVD occurrences (Uusitupa M, 2016). Moreover, recent studies have demonstrated that the use of more modern antidiabetic drugs can significantly

lower the risk of CVD in T2DM patients with high CVD risk (Sattar N, et al. 2021). A significant obstacle to preventing CVD has been identified as these factors (Lee CL, et al. 2024). Yet, people with T2DM can avoid CVD using a few useful measures (Pagidipati NJ, et al. 2023).

3.1.4. Genetic contribution to T2DM and CVD

Individual risks for T2DM and CVD are a reflection of the intricate interactions between lifestyle choices and genetic susceptibility (Lu Y, et al. 2021). While estimates of the general population's heritability of T2DM and CVD vary greatly, each individual's genetic contribution to the difference in risk can be estimated to a degree of about 30% (Strawbridge RJ, et al 2018). It is mainly unknown if T2DM disrupts pathways linked to the pathophysiology of CVD or if common risk factors predisposing to both diseases include shared genetic components (De Rosa S, et al 2018). Despite extensive efforts to discover common genetic backgrounds through genome-wide association studies (GWAS), only a small number of strong shared loci have been found between T2DM and CVD (Mahajan A, et al 2018). Important genes associated with CAD have been investigated using GWAS that were stratified by diabetes status with the aim of identifying genetic determinants of CAD in people with T2DM (Jiang Y, et al 2021). Strong shared loci between CAD and T2DM are found at chromosome 9p21.3, close to the CDKN2A and CDKN2B genes, according to previous GWAS for T2DM and CAD (Lu Y, et al 2021). A set of single nucleotide polymorphisms (SNPs) in linkage disequilibrium close to the IRS1 locus (that mediates physiological activities by relaying signals from insulin receptors to downstream signalling pathways) have been found in previous GWAS and are linked to T2DM, elevated blood pressure, reduced body fat percentage, decreased waist-hip ratio, and decreased HDL cholesterol (Nasykhova YA, et al 2020).

3.2. PARAOXONASE 1 (PON1)

3.2.1. Introduction to PON1

A number of theories have been put forth to explain HDL's antiatherogenic qualities. Of them, PON1 has drawn the most attention. PON1 is a 354 amino acid protein with a 43 kDa molecular mass (Primo-Parma et al., 1996). PON1 is a member of the same protein family as PON2 and PON3. Human chromosome 7(q21.22) contains the genes encoding for the PON family (Primo-Parma et al., 1996). PON1 is produced in the liver and some of it is secreted into the plasma, where it forms a strong hydrophobic connection with HDL particles via the N-terminal hydrophobic peptide (Sorenson et al., 1999). Human PON2 is widely expressed and present in the placenta, small intestine, spleen, stomach, testis, liver, kidney, and heart, among other tissues (Ng et al., 2001; Ng et al., 2005). Human PON2 message is found in artery wall cells such as smooth muscle, endothelium, and macrophages, in contrast to human PON1 message (Ng et al., 2001). By western blot, PON2 protein cannot be found in HDL, LDL, or cell supernatants; instead, it appears to be intracellular and linked to membrane fractions (Ng et al., 2001; Ng et al., 2005). PON2 and PON3 do not have any paraoxonase activity as a result. PON's lactonase activity has garnered a lot of interest lately. Hydrolyzing aromatic and long-chain aliphatic lactones is a shared capability of all PON family proteins (Draganov and B. N. La Du, 2004; Draganov et al., 2005). Increased PON1 lipo lactonase activity has been shown to be linked to PON1's stimulatory effect on HDL-mediated macrophage cholesterol efflux (Rosenblat et al., 2006). Several organophosphorus pesticides' active metabolites can be hydrolyzed by PON1 (Costa et al., 2003a). According to Durrington et al. (2001), PON1 serves as the primary defence of the nervous system against the neurotoxicity of organophosphates that enter the bloodstream. The most recent studies have concentrated on the PON1's function in HDL's cardioprotective effects. PON1 is an antioxidant enzyme that breaks down the byproducts of lipid peroxidation to shield lipoproteins and cell membranes from the damaging

effects of ROS (Adamczyk et al., 2012). Similar to PON1, human PON2 and PON3 have also been demonstrated to stop LDL from being oxidatively modified by cells (Reddy et al., 2001; Ng et al., 2001). According to Rosenblat et al. (2005), HDL-associated PON1 has been shown to act on macrophage phospholipids to form lysophosphatidylcholine, which in turn stimulates HDL binding and HDL-mediated macrophage cholesterol efflux via the ABCA1 transporter, potentially attenuating the development of atherosclerosis.

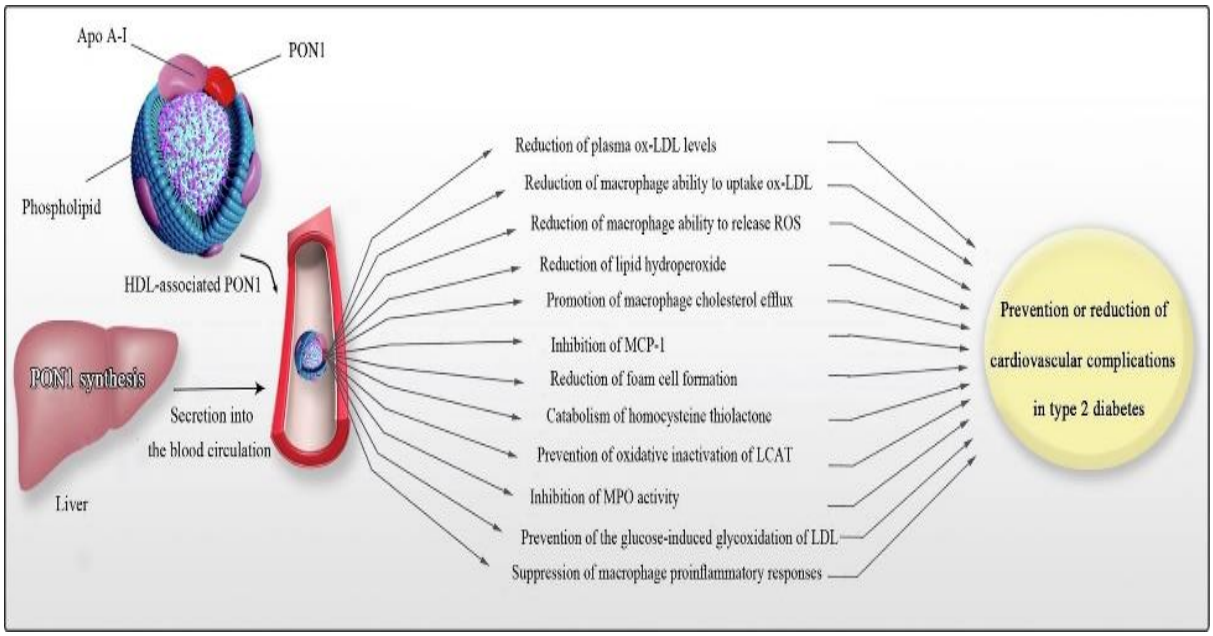


Fig.1. Protective mechanisms of HDL-associated PON1 in reducing cardiovascular complications in type 2 diabetes. The figure illustrates how PON1, associated with HDL, plays a significant role in reducing cardiovascular risks by lowering LDL levels, inhibiting oxidative stress, preventing foam cell formation, and inhibiting various pro-inflammatory responses (Shokri, et al. 2020).

3.2.2. Enzymatic Activities of Human Serum PON1.

Aldridge (1953b) first showed that PON1 hydrolyzes phosphoric acid triesters, including derivatives of organophosphate (OP) such paraoxon. This led to the nomenclature. Aromatic esters like phenyl acetate are also hydrolyzed by PON1. When observing the activity of an enzyme, namely paraoxonase and arylesterase activity, the two substrates most frequently

utilised are paraoxon and phenyl acetate (Tang WW, et al. 2012). According to (Taler-Verčič A, et al. 2020), PON is also known to hydrolyze the following substrates: diazinon, chlorpyrifos, soman, sarin, 4-nitro phenyl acetate, and soman. The ancestral enzyme activity is lactonase, as demonstrated by thorough tests conducted against several lactone substrates. Oxidised fatty acid derivatives, homocysteine thiolactone, homoserine lactones, and statin medications are among the physiologically significant lactones that PONs metabolise (Harel et al., 2004; Teiber et al., 2004; Gaidukov and Tawfik, 2005). By designating PONs as lactonases, four distinct areas of interest are added to the list of possible physiological substrates. First, PON1 is primarily linked to lipid peroxidation through 5-HETEL and 4-HDOHE, which are structurally related to lipoxins and are byproducts of the enzymatic and nonenzymatic oxidation of arachidonic acid and docosahexaenoic acid, respectively (Gaidukov and Tawfik, 2005). Both lipoxins and Ox-LDL have strong inflammatory properties, and they could be the substrate that connects PON1 to atherosclerosis, an inflammatory condition (Kotur-Stevuljević J, et al. 2020). Second, it is associated with oxidative stress and vascular disease, both of which are innate to PON1 and PON3's homocysteine thiolactonase (HCTL) activities (Bhattacharyya T, et al. 2008, Perła-Kajá; n J, et al. 2018). Third, homoserine lactones are significant chemicals linked to bacterial quorum sensing and PON2's metabolism of it; this suggests that PONs have an immune function (Gaidukov and Tawfik, 2005).

3.2.2.1. The Activity and Active Site for Arylesterase Hydrolysis

The part of PON1 responsible for its arylesterase action has a special area called the active site with a catalytic triad made up of three important amino acids: serine, histidine, & aspartate (Rosenblat M, et al.2011). These amino acids are key players in helping with the enzyme's actions; they stabilize what's called the transition state and help break down the ester bond in aryl esters (Doneva-Basheva K, et al. 2013). Now, surrounding this active site is a lid domain that's very essential for getting substrates to bind right & for catalysis to happen smoothly

(Rosenblat M, et al. 2006). It makes sure that everything is lined up perfectly for a successful enzymatic reaction (Taha NM, et al. 2016). PON1's active site has been designed to work with different kinds of aryl esters. Its capability to break down various substrates like phenylacetate comes from its specific interactions between substrate molecules & the roles of residues within the active site (Khersonsky O, et al. 2005). There's also a neat hydrophobic pocket within the enzyme that helps bind aromatic substrates better. This pocket grabs hold of the aromatic ring on the ester and positions it just right for hydrolysis, making everything more efficient (Mogarekar MR, et al. 2013).

PON1 is really famous for its arylesterase activity. This means it can break down esters like phenylacetate hydrolysis (Blaha-Nelson D, et al. 2017). This action is super important because it helps detoxify harmful substances & has antioxidant benefits (Linton MF, et al. 2019). So, arylesterase activity means how well the enzyme can breakdown of aryl esters—these are that have an aromatic group attached an acyl group (Khersonsky O, et al. 2005). In the hydrolysis process, PON1 works by splitting the ester bond found in substrates such as phenylacetate (Tavori H, et al. 2009). By doing this, PON1 helps lower oxidative stress by breaking down bad esters that could cause harm (Aviram M, 2004). PON1's ability to perform arylesterase activity makes it great at defending against oxidative damage to lipoproteins (Khosravi M, et al. 2019). This protective role is very important for cutting down the chances of getting atherosclerosis & other diseases related to oxidative stress (Mohammed CJ, et al. 2022). If some variations are in their arylesterase activity from PON1, it changes how likely they are to face cardiovascular diseases or other health issues. For example, lower arylesterase activity linked to genetic changes has been associated with a higher risk of coronary artery disease (Costa LG, et al. 2002).

Genetic variations, like the Q192R change, can really shake things up when it comes to how well PON1 does its arylesterase activity (Kowalska K, et al. 2015). These variants might

change how the active site looks and works, affecting its efficiency at breaking down aryl esters for example the R192 variant shows higher arylesterase activity than the Q192 version (Hassan MA, et al .2013). This difference could seriously play a role in whether someone might develop health problems related to oxidative stress or issues with lipid metabolism disorders (Wysocka A, et al. 2019).

3.2.2.2. Activity and active site for Lactonase hydrolysis

Lactonase activity is essential for the hydrolysis of lactones—cyclic esters made from hydroxycarboxylic acids is exhibited by PON1 (Taler-Verčič A, et al.2020). This lactonase function is important for lipid metabolism modulation and for detoxifying toxic chemicals (Ferretti G, et al. 2012). Many lactones, particularly those linked to atherosclerosis and other metabolic diseases, are broken down by lactonase activity (Meneses MJ, et al. 2019). PON1's Active Site for Lactonase Hydrolysis is made up of catalytic trio of serine, histidine, and aspartate residues is present in the active site of PON1 that is involved in lactonase hydrolysis (Muthukrishnan S, et al. 2012). This trio is necessary for the hydrolysis reaction because it stabilizes the transition state and makes the nucleophilic attack on the lactone ring easier (Ben-David M, et al. 2012). The hydrophobic pocket that holds the lactone substrate is part of the active site architecture of the enzyme (Hu X, et al. 2009). The exact arrangement of active site residues affects the hydrolysis reaction's specificity and efficiency by aligning the substrate for the best possible contact and catalysis (Taler-Verčič A, et al.2020).

PON1's lactonase activity is influenced by the Q192R polymorphism (Taler-Verčič A, et al.2020). Comparing the R192 version to the Q192 variant, the former often shows higher lactonase activity (Ferretti G, et al. 2012). The ability of the enzyme to hydrolyse lactones is impacted by this variation, which may also have an effect on other metabolic processes (Hernández-Díaz Y, et al. 2016). L55M Polymorphism has a smaller impact, and also

influences lactonase activity (Vavlukis M, et al. 2022). Changes in this location can impact the enzyme's ability to bind substrate and its catalytic effectiveness, which can impact how well it protects against oxidative stress and related illnesses (Murillo-González FE, et al.2020).

Differences in lactonase activity can affect a person's vulnerability to conditions including diabetes and cardiovascular illnesses (Gugliucci A, et al. 2015).

3.2.3. Genetic variations of PON1

The gene PON1 is known for having more than 400 single-nucleotide polymorphisms (SNPs), making it a highly polymorphic gene. Certain variations in the genetic makeup of an individual, called polymorphisms, can impact the activity and quantity of the enzyme PON1. Research has suggested that about 60% of changes in PON1 are linked to these genetic variations (Mahrooz A, 2016). Moreover, these polymorphisms can also affect the efficiency of splicing and polyadenylation and also the binding of transcription factors (Costa LG, et al. 2013). There are four known functional single-nucleotide polymorphisms (SNPs) in thePON1 gene that impact PON1 levels. Among these, two are located in the coding region, namely Q192R andL55M, while the other two are in the promoter sequence, namely 162A/G and 108C/T (Kim DS, et al. 2013). The rs662 polymorphism, also known as the Q192R, occurs when the amino acid glutamine (Q) is replaced by arginine (R) in codon 192. This variation has a significant impact on the enzyme's ability to catalyze substrates like paraoxon, but not phenylacetate (Mackness M, et al. 2015). Allozyme R is more effective in breaking down paraoxon, while phenylacetate is hydrolyzed equally well by both allozymes (Zargari M, et al. 2016). Allozyme Q is more efficient than R in hydrolyzing nerve agents' sarin and soman, with a 1.5 to 3.5 time's greater effect (Furlong CE, 2008). Salt has a greater effect on the R allozyme than on the Q allozyme. Previous research has found that the allozymes resulting from this polymorphism have different protective effects on LDL oxidation. People with allozyme Q are more protected against LDL

oxidation compared to those with allozyme R. Furthermore, it has been shown that allozyme Q has three times less tendency to bind to HDL than allozyme R, which could affect the enzyme's stability and function (Zargari M, et al. 2016).

3.2.4. Antiatherogenic roles of PON1

HDL-C levels demonstrate a strong inverse relationship with atherosclerosis risk, as it is an atheroprotective lipoprotein (Variji A, et al. 2019). Most studies have focused on the pivotal role of HDL in the reverse cholesterol transport process. However, HDL is a lipoprotein with pleiotropic effects, and recent research has explored other mechanisms through which HDL is involved in atherosclerosis. These mechanisms include anti-inflammatory and antioxidative effects, as well as the improvement of endothelial function (Shah PK, 2010). In recent years, some studies have suggested that the functionality of HDL (High-density lipoprotein) is more important than its mass and that the concentration of HDL-C (cholesterol carried by HDL) alone may not always accurately reflect the HDL functionality, and may not provide significant clinical benefits. Further investigations have shown that changes in HDL proteome, particularly the enzyme components of HDL, can impact its functionality, and are also associated with an increased risk of coronary artery disease (CAD). These findings could potentially lead to the development of therapeutics targeting the HDL proteome (Annema W, et al. 2016). Patients with CAD may experience negative effects on endothelial HDL functionality due to increased production of malondialdehyde in the endothelial HDL. This increased production is a result of reduced PON1 activity. Guns et al. discovered that transferring the PON1 gene can help to improve the oxidative stress of the vascular wall, endothelial function, and Ca2+ homeostasis of smooth muscle cells in segments that already have atherosclerosis. However, they found that these improvements are not related to the effect of PON1 on the size of the plaque (Guns PJ, et al. 2008)). According to a study by Younis et al., HDLs (high-density lipoproteins) that have higher paraoxonase 1 (PON1) activity are more effective in preventing the glucose-induced

glycoxidation of LDL (low-density lipoproteins) than those with lower PON1 activity (Younis NN, et al. 2013). However, the role of PON1 in this aspect is still not extensively studied, especially in individuals with diabetes mellitus. Additionally, the glycation of PON1 may contribute to inducing endothelial dysfunction by causing endoplasmic reticulum stress (Yu W, et al. 2017). One of the roles of PON1 in atherosclerosis is related to the breakdown of homocysteine thiolactone (HTL), a toxic metabolite of homocysteine (Hcy). PON1 can hydrolyze HTL and reduce N- homocysteine-protein accumulation, which can contribute to the enzyme's cardio protective function (Jakubowski H, 2010).

3.2.5. PON1 in Diabetes Mellitus

Type 2 diabetes is characterised as a long-term hyperglycemic illness caused by a relative insulin shortage (American Diabetes Association, 2020). There are two stages to the disorder's development. The first is when skeletal muscle, adipose, and hepatic tissue become resistant to the effects of insulin (Hannon TS, et al. 2015). These people have multifactorial insulin resistance, which is caused by a combination of inherited and acquired predispositions, most frequently obesity (Saltiel AR, et al. 2001). In the prediabetic stage, there is an increase in insulin secretion as a first reaction to insulin resistance (DeFronzo RA, et al. 2009). Hyperglycemia results in the second stage, which happens when hyperinsulinemia can no longer make up for insulin resistance (Kim SH, et al. 2008). The cardiovascular metabolic syndrome includes risk factors that accelerate the atherosclerotic process in diabetes, including hypertension, hyperinsulinemia, atherogenic dyslipidemia, coagulation abnormalities, platelet and endothelial dysfunction, and microalbuminuria (Srikanth S, et al. 2016). Long-term exposure to hyperglycemia is now understood to play a significant role in the development of atherosclerosis and other diabetic problems (Aronson, 2008). Serum PON1 activities (arylesterase) in individuals with diabetes were found to be significantly lower than those in healthy control subjects (Rosenblat et al., 2008). This finding suggests that the cause of the

lower serum PON1 activities in diabetic patients may be oxidation and/or glycation, or replacement by serum amyloid A (SAA) associated with HDL, under inflammatory conditions (Kontush, 2008). Reduced serum PON1 activity has been linked to increased atherogenesis in both familial hypercholesterolemia and diabetes mellitus. Lipid peroxidation may be a contributing factor to arterial wall damage due to the acceleration of oxidative stress, as noted by (Sies H, 2015).

3.2.6. PON1 in cardiovascular risk

Coronary heart diseases and other indications of atherosclerosis are the most significant risk factors in patients with type 2 diabetes (Pagano E, et al. 2021). Increased oxidative stress due to the overproduction of reactive oxygen species will lead to severe risk factors for cardiovascular diseases and in most cases will cause death (Kayama Y, et al. 2015). PON1is found to be an antioxidant and athero-protective due to the association of high-density lipoprotein thatprevents oxidation of low-density lipoprotein (Chistiakov DA, et al. 2017). A study by Lavi S, et al. demonstrated that oxidative stress and predicting possible cardiovascular risk are influenced by PON1 Q192R polymorphism, and PON1 activity and also reported from previous studies that have shown PON1 192Q alleles correlated with the endothelial dysfunction in patients with established coronary artery disease andperipheral vascular disease (Lavi S, et al. 2008). Coronary artery disease results in mortality in developed countries (Hayward RA, et al. 2010). Increased lipid peroxidation is correlated with the progression of atherosclerosis. The high activity of PON1 protects against lipid peroxidation and coronary artery disease (Gugliucci A, et al. 2015). According to Mendonca et al the data suggest the presence of a synergic effect of the PON1 RR192 genotype with a reduced level of antioxidant ability and High-density lipoprotein deficient is susceptible to the risk of coronary artery disease (Mendonca MI, et al. 2010)

4. MATERIAL AND METHODS

Research setting:

Department of Biochemistry & Central Research Facility

Santosh Deemed to be University, Ghaziabad -201009

Ethical considerations:

The necessary approval was obtained to conduct the study from Institutional Ethical committee (IEC). Patients were given an explanation regarding the study protocol. They were informed about the confidentiality clause as well as optional participation.

Period of study: 12 Months

Study design: Cross sectional study

Sample size: n= 60 (30 Type 2 Diabetic and 30 healthy control subjects)

Inclusion criteria:

1. Type 2 Diabetes Mellitus Patients (newly diagnosed with T2DM)
2. People those who are living since 15 years in and around Ghaziabad
3. Patients who will give consent for enrollment in the study

Exclusion criteria:

1. Other types of diabetes (e.g., type 1 diabetes, gestational diabetes)
2. Chronic liver disease
3. Chronic kidney disease
4. History of cancer
5. Pregnancy and lactation
6. Cardiovascular disease

Data collection:

A standard questionnaire which included the questions regarding personal data i.e.name of the patient, sex, residence, age, occupation, etc were filled. Before taking blood samples proper consent was taken from diabetic as well as non-diabetic subjects. History was taken and the record was maintained.

Sample collection:

Fasting venous blood samples were collected from 30 T2DM subjects and 30 healthy controls subjects. The blood samples were separated into following vials- sodium fluoride vials for fasting glucose estimation, red topped serum tubes for lipid profile and ELISA and EDTA vials for DNA extraction. The blood samples in EDTA vials were stored at -20ºC till DNA was extracted.

4.1. Biochemical parameters

From the collected blood samples serum and plasma were separated by low speed centrifugation for biochemical analysis. Fating Blood glucose, HbA1C and serum lipid profile (Total Cholesterol- TC (Cat no: E-BC-K109-S), Triglycerides-TGL (Cat no: E-BC- K238), High Density Lipoprotein- HDL (Cat no: E-BC- K222-S) from (Elabsciences).Low Density

Lipoprotein-LDL and Very Low Density Lipoprotein-VLDL) were measured by using enzymatic method/kit as per manufacturer's protocol. PON1 concentration was measured using ELISA kit procured from manufacturer's protocol ELISA kit Cat# E-EL-H2298 (Elabsciences Biotechnology Inc, USA).

4.2. DNA isolation from human blood

Consumables: Sterile Syringe, fresh blood samples, Vaccutainers (10 ml), micro-pipettes, micro-tips, cotton, tissue paper, disposable gloves, eppendorf (1.5 ml).

Collection of Samples

Blood samples collected from all the subjects and stored in EDTA for DNA isolation.

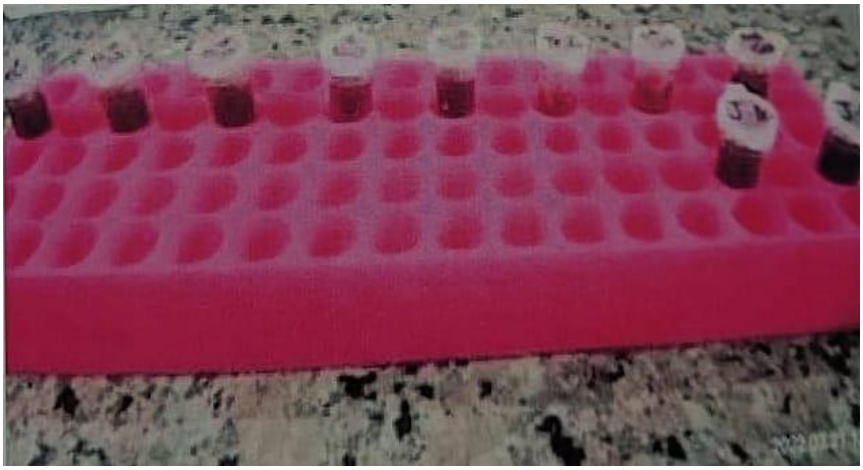


Fig. 2. Blood samples

4.3. Method of DNA Isolation from human blood

Chemicals Required:

QIamp DNA Blood mini kit (Diagen, USA, Cat No. 51104) and Fianol. Genomic DNA was isolated by using QAamp DNA Blood mini kit (Olagen, USA, Car No. 51104) following protocol was used as per manufacturer's instruction.

Purification of DNA from Whole Blood

All centrifugation steps was carried out at room temperature and all the samples, Buffer AE or distilled water was brought to room temperature (15-25°C).

1. 20 µl QIAGEN Protease (or proteinase K) was pipetted into the bottom of a 1.5 ml microcentrifuge tube.
2. 200 µl sample was added to the microcentrifuge tube. Use up to 200 µl whole blood in 200 µl PBS.
3. 200 µl Buffer AL was added to the sample. Mixing was done by pulse-vortexing for 15 sec. The sample and Buffer AL was mixed thoroughly to yield a homogeneous solution for efficient lysis
4. The sample and Buffer AL was Incubated at 56°C for 10 min.
5. To remove drops from the inside of the lid centrifugation was done the 1.5ml microcentrifuge tube
6. 200 µl ethanol (96-100%) was added to the sample, and mix again by pulse-vortexing for 15 s. After mixing, we again did centrifuge 1.5 ml microcentrifuge tube to remove drops from the inside of the lid.
7. Mixture was applied carefully to the QlAamp Mini spin column (in a 2 ml collection tube) without wetting the rim. The cap was closed, and centrifuge at 6000 x g (8000 rpm) for 1 min. We had put the QIAamp Mini spin column in a clean 2 ml collection tube, and the filtrate was discarded. Each spin column was closed carefully to avoid aerosol formation during centrifugation.

8. The QlAamp Mini spin column was opened carefully and add 500 ul Buffer AW1 without wetting the rim. The cap was closed and centrifuge at 6000 x g (8000 rpm) for 1 min. We placed the OlAamp Mini spin column in a clean 2 ml collection tube, and discard the filtrate.
9. We carefully opend the QIAamp Mini spin column and 500 ul Buffer AW2 was added. The cap was closed and centrifuged at full speed (20, 000 x g; 14, 000 rpm) for 3 min.
10. It was recommended that the QIAamp Mini spin column was placed in a new 2 ml collection tube and the old collection tube with the filtrate was discarded. Centrifugation was done at full speed for 1 min.
11. Now the QIAamp Mini spin column was placed in a clean 1.5 ml microcentrifuge tube, and the collection tube containing the filtrate was discarded. The QIAamp Mini spin column was opened carefully and 200 µl Buffer AE or distilled water was added. Incubation done at room temperature (15-25°C) for 1 min, and then centrifuge at 6000 x g (8000 rpm) for 1 min.
12. Incubation was done at room temperature for the QIAamp Mini spin column loaded with Buffer AE or water for 5 min before centrifugation to increases DNA yield.
13. A second elution step with a further 200 µl Buffer AE had increased the yields by up to 15%.
14. DNA was eluted in Buffer AE and stored at -30 to -15°C for long-term storage.

4.4. Quantitative and Qualitative analysis of extracted genomic DNA:

To check the quality of DNA, isolated DNA was further studied on 1.0 % agarose gel and UV spectrophotometer.

a. Agarose Gel Electrophoresis

Materials:

a. Agarose

b. TAE buffer (1X) or TBE buffer

c. EtBr [Ethidium Bromide]

d. Loading dye/Tracking dye (6X)- [Bromophenol Blue or Xylene Cynol]

Instruments:

Balance, Micro-pipette, Microwave, Electrophoresis Unit (Biorad, USA), Transilluminator (Biorad, USA) DNA is a negatively charged molecule because of its phosphate group and under electric field it moves towards anode through a matrix of agarose.

Procedure:

- 1 gm agarose powder was weighed and added to 100 ml of 1X TAE buffer, then heated in microwave oven until completely melted.
- EtBr [final concentration 0.5µg/ml was added to the melted agarose when temperature of gel is bearable on hands.
- Melted agarose was poured on pre-placed gel tray.
- Once the gel was solidified, the comb is removed, using care not rip the bottom of the well.
- 2µl of each DNA sample was added to l µl tracking dye and gently mixed with micropipette.
- The lid was closed and power leads were placed on the apparatus. 60V current was applied on the buffer tank.

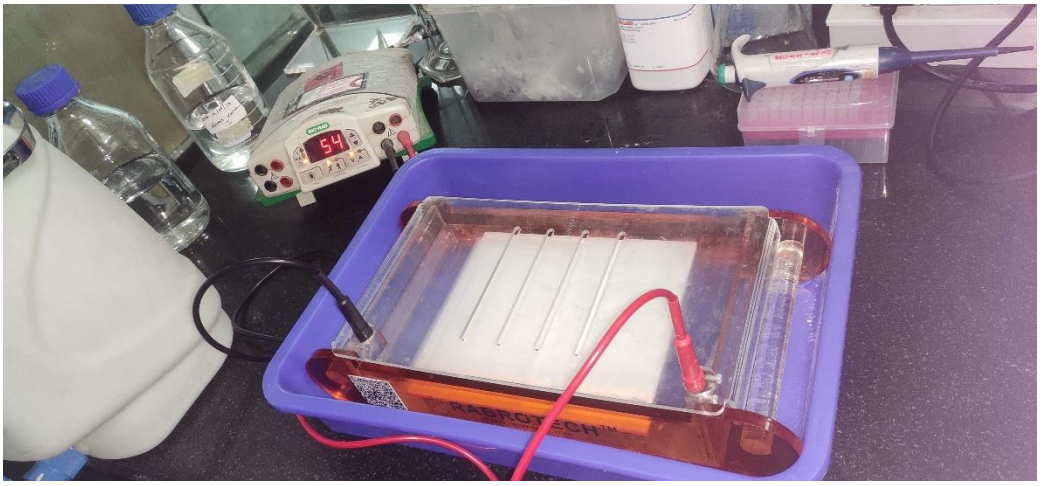


Fig. 3: Horizontal Gel Electrophoresis for DNA samples

- The distance DNA had migrated in the gel and was judged by visually monitoring the migration of tracking dye.
- Bromophenol blue or Xylene cynol dyes migrated through Agarose gels towards anode.
- When adequate migration had occurred the power supply was switched off and the gel was carefully taken out from the buffer tank place in UV transilluminator.
- As the fluorescent dye EtBr interecalates between bases of DNA, the bands of respective DNA fluoresces with a pattern of gel was visible.

Primers used for polymorphic study:

S.no.	**Gene**	**Primer Sequences**
a.	PON1 (F1)	5’-CCCAAATACATCTCCCAGGATT-3’
b.	PON1 (F2)	5’-CCCAAATACATCTCCCAGGATC-3’
c.	PON1 (Reverse)	5’-TAAGGATTGTATCGGCAGGAC-3’

d.	PON1 (E)	5’-CCACTACATTTCAGAGAGTTCAC-3’

Table.1: Primers Forward & Reverse with probes (F1 and F2)

Analysis of Q192R (rs662) PON1 Polymorphism

The PON1 polymorphism at position Q192R was determined by using the following oligonucleotide primers and probes for Q192R; 5’- CCACTACATTCAGAGAGTTCAC-3’ forward and 5’-TAAGGATTGTATCGGCAGGAC-3’ reverse; 192Q; 5’CCCAAATACATCT CCCAGGATT3’ (F1) and 192R: 5’CCCAAATACATCTCCCAGGATC-3’ (F2).

ARMS-PCR technique was used to study the SNP changes in the PON1 gene. In this technique reaction was formed in a tube. The ARMS-PCR technique was used to determine the presence of A>G point mutation with primer pairs.

A proliferation fragment was seen in all samples as an indicator of the accuracy of PCR. The expected results of ARMS-PCR in this study included the normal genotype AA, as well as the AG and GG mutant genotypes. After PCR, the products were separated on an agarose gel by electrophoresis and then bands were observed using ultraviolet visualization.

Amplification Refractory Mutation System Polymerase Chain Reaction (ARMS- PCR)

The ARMS-PCR (Amplification Refractory Mutation System-PCR) technique is used to detect specific alleles or mutations. This method uses allele-specific primers to amplify only the target allele, differentiating between the wild-type and mutant alleles. The ARMS-PCR technique was employed to determine the presence of the A and G alleles at the Q192R position in the PON1 gene. The PCR reactions were performed in a final volume of 20 μL, which included:

Fig. 5: PCR Cycler

a. Materials for PCR:

- 25–50 ng of genomic DNA
- 0.2–0.5 µM of each primer
- 10 µL of 2X PCR master mix (containing Taq DNA polymerase, dNTPs, MgCl2, and buffer)
- Nuclease-free water to the final volume

Each DNA sample was subjected to two separate PCR reactions: one with PON1 (F1) for the A allele and one with PON1 (F2) for the G allele.

b. Concentration of Components for 20µl of Reaction:

Component	**Stock Concentration**	**Final Concentration**	**Volume**
DNA Template	**200ng/µl**	**100ng-1µl**	**1**µl
Buffer	10X	1X	**2**µl
dNTPs	10mM of each	200µM of each	**0.4**µl
Taq Polymerase	3U/ µl	1 Unit	**0.33**µl
Primers	100pm/µl	0.05µM	**2**µl

MgCl2	20mM	1.5mM	**-**
Nuclease free water (NFW)	…….	To make Volume upto 20 µl	**14.27**µl
			Total 20µl

Table 2: Concentration of components for PCR

PCR CYCLING CONDITIONS

The amplification was carried out in a thermo cycler under the following cycling conditions:

- Initial denaturation at 95°C for 5 minutes

35 cycles of:

- Denaturation at 95°C for 30 seconds
- Annealing at 57°C for 30 seconds
- Extension at 72°C for 30 seconds
- Final extension at 72°C for 5 minutes

The PCR products were then held at 4°C until further analysis.

Gel Electrophoresis

The amplified products were analyzed using 2% agarose gel electrophoresis. Gels were prepared by dissolving 2 g of agarose in 100 mL of 1X TBE buffer and stained with ethidium bromide (0.5 µg/mL). A 5 µL aliquot of each PCR product was mixed with 1 µL of 6X loading dye and loaded into the wells of the gel alongside a 100 bp DNA ladder as a size reference. Electrophoresis was performed at 100 V for 45 minutes in 1X TBE buffer.

Visualization and Genotyping

The gels were visualized under ultraviolet (UV) light. The presence of a specific PCR product indicated the presence of the corresponding allele.

Samples were classified as homozygous A/A, homozygous G/G, or heterozygous A/G based on the pattern of bands observed.

c. Measurement of PON1 concentration in serum using ELISA method:

The concentration of PON1 was measured in the serum of patients and controls using manufacturer's protocol (Elabsciences Biotechnology Inc, USA).

ELISA Protocol:

Test Principle:

This ELISA kit is based on the Sandwich-ELISA principle. The micro ELISA plate were pre-coated with an antibody specific to Human PON1. Standards or samples were added to the micro ELISA plate wells and combined with the specific antibody. Then a biotinylated detection antibody specific for Human PON1 and Avidin-Horseradish Peroxidase (HRP) conjugate were added successively to each micro plate well and incubated. Free components were washed away. The substrate solution was added to each well. Only those wells that contain Human PON1, biotinylated detection antibody and Avidin-HRP conjugate was appeared blue in color. The enzyme-substrate reaction was terminated by the addition of stop solution and the color turned yellow. The optical density (OD) was measured spectrophotometrically at a wavelength of 450 nm ± 2 nm. The OD value was proportional to the concentration of Human PON1. The concentration of Human PON1 in the samples was calculated by comparing the OD of the samples to the standard curve.

b. Assay Procedures

1. 100µL standard or sample were added to the wells followed by Incubation for 90 min at 37°C.
2. After incubation the liquid was discarded, 100µL Biotinylated Detection Ab working solution was immediately added to each well followed by Incubation for 60 min at 37°C.
3. Aspiration and washing of the plate was done for 3 times.
4. 100µL HRP conjugate working solution was added and incubate for 30 min at 37°C. Aspiration and washing of the plate was done for 5 times.
5. 90µL Substrate Reagent was added followed by incubation for 15 min at 37°C.
6. 50µL stop solution was added finally to stop the reaction.
7. Absorbance of the plate was noted at 450nm immediately and Calculation of the results was done.

S.NO.	CONC (ng/ml)	OD1	OD2	AVERAGE OD	CORRECTED READING
1	10	5.1763	5.1969	5.1866	5.1866
2	5	2.3033	2.7851	2.5442	2.5417
3	2.5	1.3004	1.88	1.5902	1.5877
4	1.25	0.7064	0.6901	0.69825	0.69575
5	0.63	0.5023	0.5369	0.5196	0.5171
6	0.32	0.164	0.2815	0.22275	0.22275
7	0.16	0.0931	0.1095	0.1013	0.0988
8	0	0.0029	0.0021	0.0025	0

Table.3. Values of absorbance for standard concentrations acquired by spectrophotometry

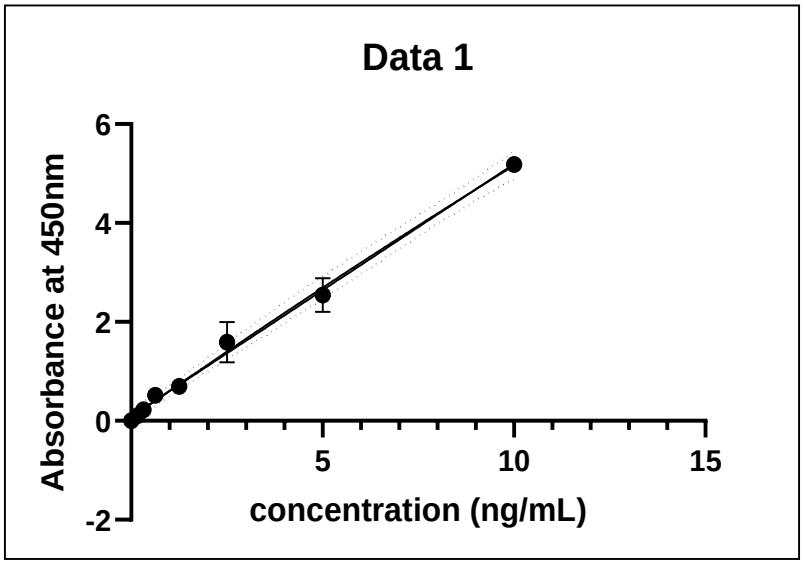


Fig. 6: Plot of concentration Vs. Absorbance for serum concentration of PON1

4.5. Statistical analysis

Data were analyzed by using IBM SPSS software version 26. The results for continuous variables were expressed as mean ± standard deviation and normally distributed variables were analyzed using student's t- test. Chi-square was used to test the association in genotypes frequency between the groups. Means of serum PON1 concentration according to different genotypes were compared using analysis of variance (ANOVA) and t-tests. Logistic regression was used to calculate the odds ratios to test the association of genotypes and T2DM, keeping

wild type as the reference. The association of Q192R polymorphism with other CVD risk factors was tested by multiple logistic regression analysis, assessing independence from potential confounders. P value less than 0.05 was considered significant.

5. RESULT

This study was performed to investigate the relationship between PON1 polymorphism and its influence on cardiovascular risk in type 2 diabetic patients without clinically diagnosed cardiovascular disease. This study involved 60 individuals including 30 control (non-diabetic) and 30 T2DM subjects. The diagnostic criterion of the American Diabetic Association (ADA, 2010) was followed to diagnose subjects with T2DM.

5.1. General Characteristic features and biochemical parameters of the study subjects

The biochemical parameters of all the study subjects were measured. The independent t-test analysis showed that the fasting blood glucose (FBG), Glycated Hemoglobin (HbA1c), body mass index (BMI), triglycerides (TG), very low density lipoprotein (VLDL) and low-density lipoprotein (LDL) levels in T2DM patients were significantly higher than control subjects ($p<0.0001$; table 4.). T2DM patients showed significantly higher level of BMI, SBP and DBP.

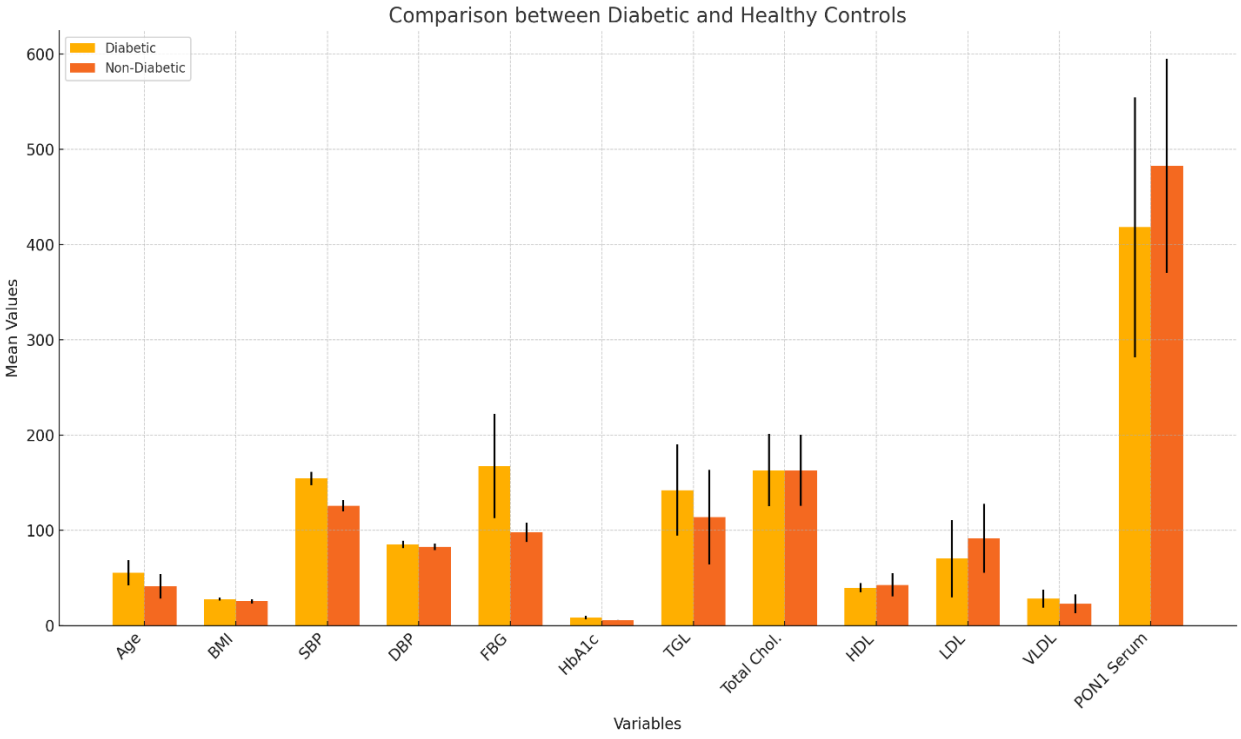
The mean of serum PON1 enzyme concentration was significantly lower in T2DM patients compared to control subjects ($p= 0.0499$).

Table.4. General Characteristics and biochemical variables of the study population

Variables	Diabetic n=(30)	Non Diabetic (n=30)	P value
Age (years)	55.36 ± 13.24	40.8 ± 12.91	<0.0001
Gender (Male/female)	13/17	15/15	
BMI	27.50 ± 1.42	25.32 ± 2.173	<0.0001
SBP	154.5 ± 6.86	125.6 ± 6.083	<0.0001
DBP	84.83 ± 4.04	82.66 ± 3.497	0.03

FBG	167.44 ± 54.53	97.72 ± 10.21	<0.0001
HbA1c	8.25 ± 1.90	5.32 ± 0.175	<0.0001
TGL	142.1 ± 47.72	113.79 ± 49.62	0.0281
Total Cholesterol	162.93 ± 37.98	162.84 ± 37.46	0.9927
HDL	39.45 ± 4.688	42.59 ± 12.24	0.1946
LDL	70.14 ± 40.61	91.429 ± 36.145	0.0362
VLDL	28.15 ± 9.31	22.51 ± 9.90	0.0267
PON1 Serum (ng/mL)	418.26 ± 136.42	482.88 ± 112.41	<0.0499

The table shows the biochemical parameters and clinical characteristics of the Type 2 Diabetes Subjects and healthy controls. The Data are shown as mean ± SD, analyzed by independent t-test, $p<0.05$ was considered as statistically significant. **BMI**: body mass index; **SBP**: systolic blood pressure: **DBP**: diastolic blood pressure; **FBS**: fasting blood sugar; **HDL**: high density lipoprotein; **LDL**: low density lipoprotein; **VLDL**: very low-density lipoprotein.



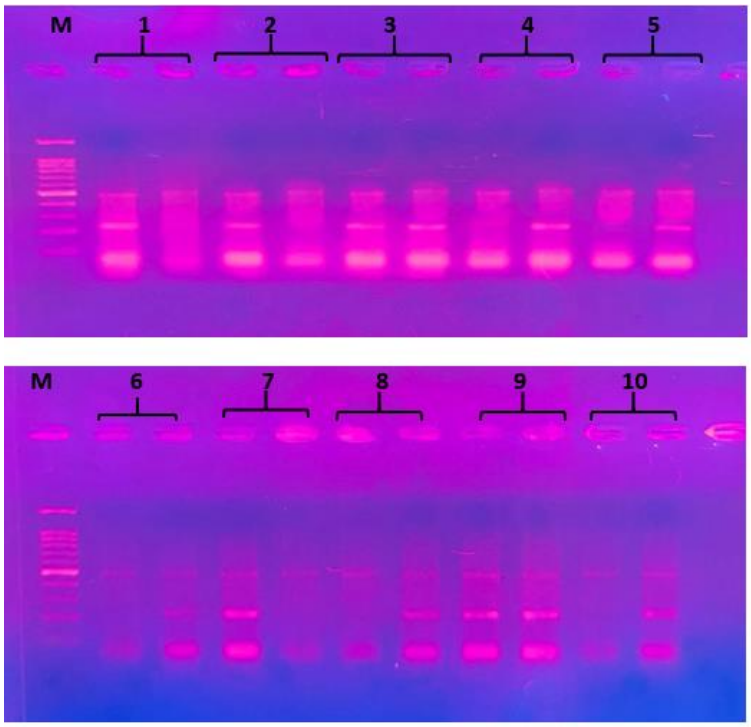
5.2. Evaluation of genomic DNA obtained from T2DM subjects and normal healthy controls

The Genomic DNA was extracted from whole blood samples of 30 T2DM subjects and 30 normal healthy controls. The genomic DNA so obtained was checked for integrity by loading 2µl of DNA on 1% agarose gel. DNA in the gel was visualized under UV light.

Determination of PON1 Q192R (rs662) single nucleotide polymorphism

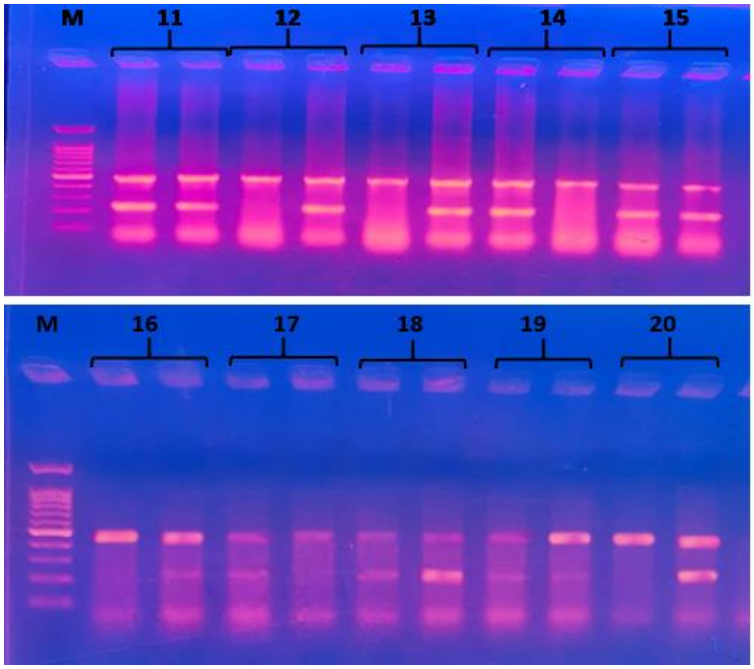
ARMS-PCR technique was used to study the SNP changes in the PON1 gene. In this technique reaction was formed in a tube. The ARMS-PCR technique was used to determine the presence of A>G point mutation with primer pairs PON1 5'- CCACTACATTCAGAGAGTTCAC-3' forward and 5'-TAAGGATTGTATCGGCAGGAC-3' reverse; 192Q; 5'CCCAAATACATCT CCCAGGATT3' (F1) and 192R: 5'CCCAAATACATCTCCCAGGATC-3' (F2).

A proliferation fragment was seen in all samples as an indicator of the accuracy of PCR. The expected results of ARMS-PCR in this study included the normal genotype AA, as well as the AG and GG mutant genotypes. After PCR, the products were separated on an agarose gel by electrophoresis and then bands were observed using ultraviolet visualization (Fig.7a, 7b, 7c for T2DM patients and 8a, 8b, 4c for healthy control).



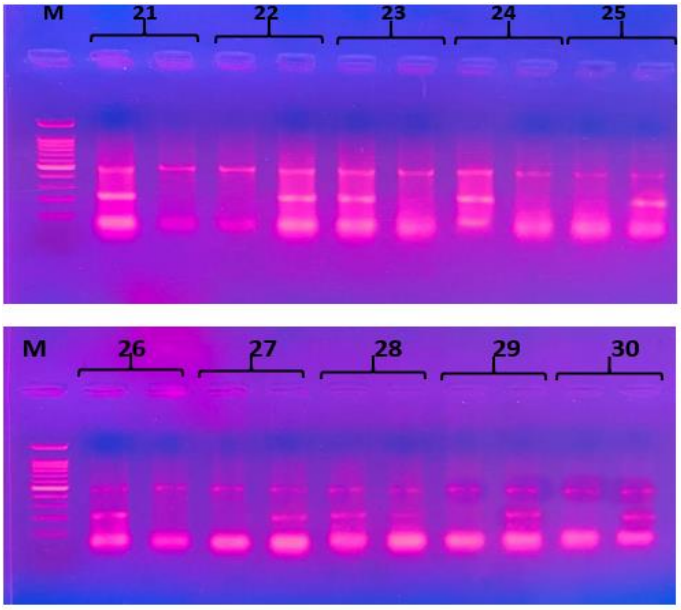
T2DM	Genotype
1	AA
2	AA
3	AG
4	GG
5	GG
6	GG
7	AA
8	GG
9	AG
10	GG

Fig.7a.



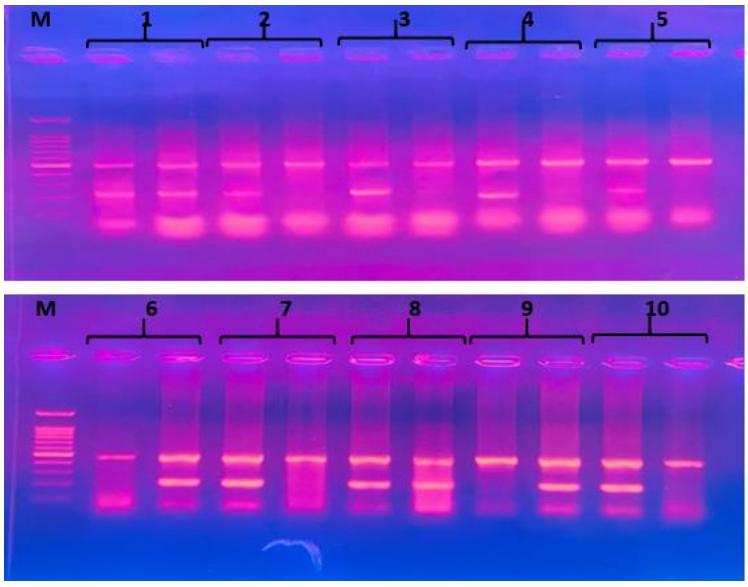
T2DM	Genotype
11	AG
12	GG
13	GG
14	AA
15	AG
16	GG
17	AA
18	AG
19	AG
20	GG

Fig.7b



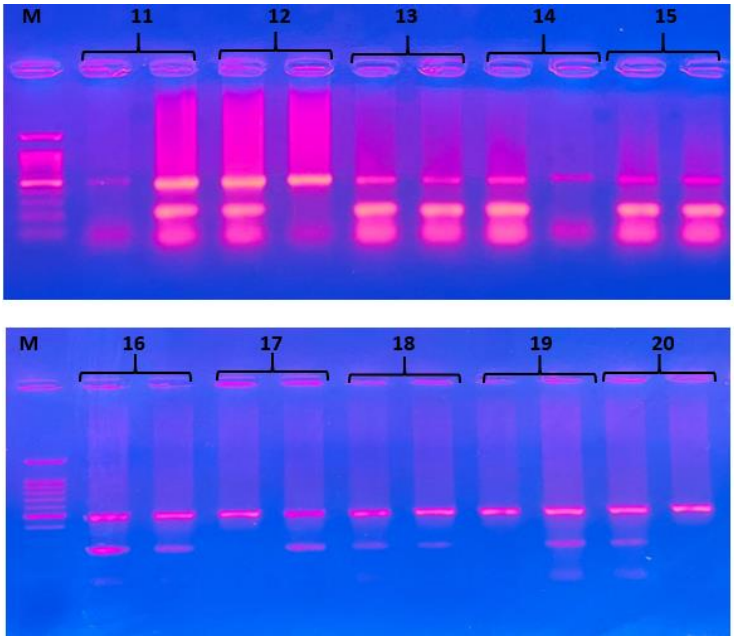
T2DM	Genotype
21	AA
22	GG
23	AA
24	AA
25	GG
26	AA
27	GG
28	AG
29	GG
30	GG

Fig.7c



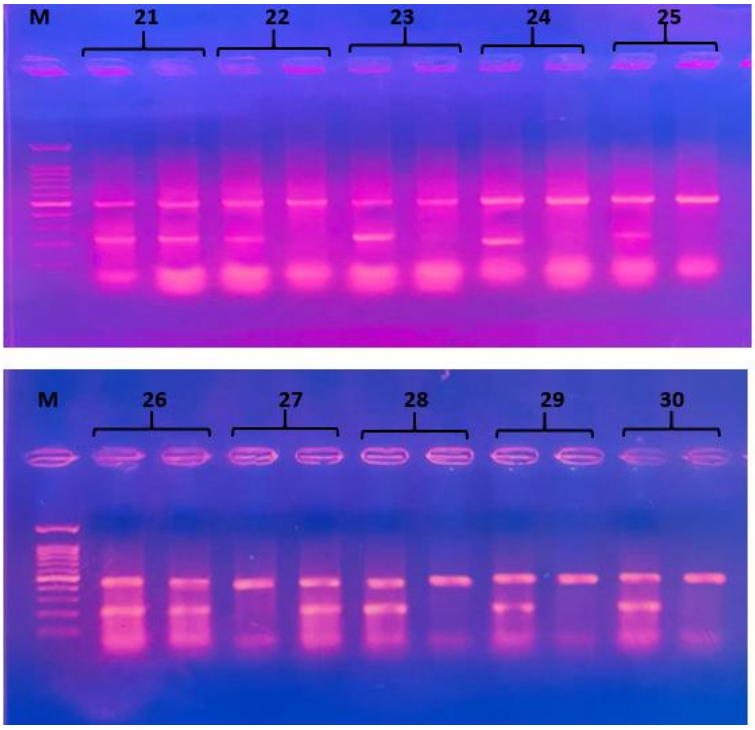
Control	Genotype
1	AG
2	AA
3	AA
4	AG
5	AA
6	AG
7	AA
8	AG
9	GG
10	AA

Fig. 8a



Control	Genotype
11	GG
12	AA
13	AG
14	AA
15	AG
16	AG
17	GG
18	AG
19	GG
20	AA

Fig.8b



Sample	Genotype
21	AG
22	AA
23	AA
24	AA
25	AA
26	AG
27	GG
28	AA
29	AA
30	AA

Fig. 8c

5.3. PON1 polymorphisms genotype and allele distribution in patients vs. controls

Analysis of genotypes and alleles distribution of Q192R in T2DM and control subjects was consistent with Hardy-Weinberg equilibrium in all studied groups and is shown in Table 5. Data revealed that the genotype and allele frequencies of Q192R were significantly different between controls and diabetic patients. Genotype 192QQ was detected in 50% of controls vs. 30% of diabetic patients, while QR and RR genotypes were more frequent in diabetic patients than in controls (23.3% and 46.6% vs. 33.3% and 16.6%, respectively). The frequency of QR genotype was not significantly higher in T2D patients than the QQ genotype (OR=1.16, 95% CI=0.32 to 4.15, P=0.81). Further, RR genotype frequency also showed a significant increase in T2D patients as compared to controls with OR's being 4.66 (95% CI=1.25 to 17.3, P=0.02). Allele Q was found in 66.6% of controls vs. 41.6% of patients, while allele R was significantly increased in T2D patients when compared to controls (58.3% vs 33.3% respectively) and it is significantly associated with the risk of T2D (OR=2.80, 95% CI=1.33 to 5.8, P=0.006) (Table 5).

Table.5. Genotypes and alleles frequency of PON1 Q192R polymorphisms in patients and control groups

	Control N=30	**Patients N=30**	**OR (95% CI)**	**P value**
Q192R				
QQ	15 (50%)	9 (30%)	Reference	
QR	10 (33.3%)	7 (23.3%)	1.16 (0.32 to 4.15)	0.81
RR	5 (16.66%)	14 (46.66%)	4.66 (1.25 to 17.3)	0.02

Allele	2n= (60)	2n= (60)		
192Q	40 (66.66%)	25 (41.66%)	Reference	
192R	20 (33.33%)	35 (58.33%)	2.80 (1.33 to 5.8)	0.006

This table shows genotypic and allele frequencies of T2DM patients vs controls for Q192R SNP. P<0.05 was considered as statistically significant.

OR = odds ratio; **CI** = confidence interval; [a] Reference category; comparison between group was performed with Chi-square test.

5.4. Effects of Q192R polymorphisms on PON1 serum enzyme concentration

The study identified that serum PON1 levels were significantly different between 192QQ genotype carriers and carriers of combined 192QR+192RR genotypes. Specifically, it was determined that the mean serum PON1 concentration in individuals with a genotype of 192QQ was higher than those with 192QR and 192RR genotypes, at 500.9 ± 73.3 ng/mL versus 382.8 ± 142.9 ng/mL, respectively shown in Table 6 . Therefore, the statistical significance of the difference detected by the P value of 0.006 suggests that this polymorphism has a major influence on the serum levels of PON1. Higher serum level of PON1 in 192QQ individuals may reflect functional differences conferred by this genotype on either the enzyme activity of PON1 or its physiological function.

Table.6. Means of serum PON1 concentration in different genotypes of Q192R polymorphisms

	Serum PON-1 mean SD (ng/mL)	**P value**

192QQ	500.9 ± 73.3	0.006
192QR + 192RR	382.8 ± 142.9	

Means of serum PON1 concentration according to different genotypes are shown in Table 6. Q192R polymorphism associated with low serum PON1 concentrations, and lowest concentrations were observed in QR and RR genotypes (p = 0.006).

5.5. Association of Q192R with CVD in T2DM patients associated with biochemical parameters

One way ANOVA test analysis was used to investigate potential correlations of 192R allele with other cardiovascular risk factors: HbA1c, BMI, SBP, DBP, TC, HDL, LDL, TG, VLDL and FBS. Then, the statistical comparisons performed among the genotypes did not present any significant differences for most parameters: the P values were as follows: SBP = 0.782, DBP = 0.543, FBS = 0.589, HbA1c = 0.419, BMI = 0.334, total cholesterol = 0.332, TG = 0.335, LDL = 0.159, HDL = 0.994, and VLDL = 0.301, respectively. These P values will indicate no statistical difference in these measurements between AA, AG, and GG genotypes. On the whole, results have shown that the genotype does not affect these metabolic and cardiovascular parameters significantly among the subjects under consideration. Probably, other causative genetic or environmental factors influencing such outcomes may be unraveled in further research.

Table.7. Biochemical and clinical characteristics of the study population according to PON1 genotype for Q192R polymorphism in T2DM patients

Variables	**AA (n=9)**	**AG (n=7)**	**GG (14)**	**P- value**

SBP(mm/Hg)	155.00 ± 6.1	152.8 ± 7.5	155 ± 7.3	0.782
DBP (mm/Hg)	83.8 ± 2.2	84.2 ± 1.8	85.7 ± 5.4	0.543
FBS (mg/dl)	163.4 ± 31.0	151.9 ± 31.2	177.7 ± 73.0	0.589
HbA1c (%)	7.6 ± 0.8	8.0 ± 1.1	8.7 ± 2.5	0.419
BMI (kg/m^2)	27.0 ± 1.1	28.1 ± 1.2	27.5 ± 1.1	0.334
Total Cholesterol (mg/dl)	178.7 ± 43.8	158.4 ± 22.4	155 ± 39.3	0.332
TG (mg/dl)	159.3 ± 55.2	123.4 ± 32.1	140.4 ± 48.2	0.335
LDL (mg/dl)	106.6 ± 33.3	95.4 ± 21.6	79.6 ± 35.8	0.159
HDL (mg/dl)	39.5 ± 4.0	39.2± 2.4	39.4 ± 5.9	0.994
VLDL (mg/dL)	31.8 ± 11.0	24.6 ± 6.4	27.5 ± 9.0	0.301

Values are mean ± SD; Genotypes were compared by one-way **ANOVA**. **BMI**: body mass index; **SBP**: systolic blood pressure; **DBP**: diastolic blood pressure; **FBS**: Fasting blood sugar; **HDL**: high density lipoprotein; **LDL**: low density lipoprotein; **VLDL**: very low density lipoprotein;

6. DISCUSSION

PON1 is an arylesterase that is produced in the liver and is bound by HDL. Because of its capacity to break down lipid peroxides and shield low-density lipoprotein from oxidation, it has been suggested to possess antioxidant and cardioprotective qualities (Bhattacharyya T, et. 2008). It is believed that oxidative alteration of low-density lipoprotein (LDL) is a primary initiating factor in the development and advancement of atherosclerosis and cardiovascular events (Hassan MA, et al. 2013). PON1 has been the subject of extensive investigation recently due to the discovery of its antioxidant qualities, including its ability to protect LDL from oxidative damage.

In the present study, we analyzed the association of PON1 Q192R polymorphism with the CVD in T2DM patients. The current investigation showed that T2D patients had a considerably higher frequency of the PON1 192 RR genotype and R allele than controls, the odds ratio (OR= 4.66, p= 0.02). It indicates an association between the risk of T2D and the PON1 Q192R polymorphism. Our diabetes patients had lower serum PON1 enzyme concentrations than controls, and the 192R allele was linked to the lower enzyme concentrations. There was also shown to be a strong correlation between the 192R allele and CVD with T2DM in accordance with a previous study done by North Indian population (Kumar R et al. 2021).

Our results on the single nucleotide polymorphism PON1 Q192R in T2DM patients are in line with those of Huo X et al. (2019), Shokri et al. (2020), and EI-Lebedy et al. (2014), who found a correlation between PON1 Q192R and cardiovascular disease, coronary artery disease, and T2DM in populations from Asian, Caucasian and Egypt.

In this study, one way ANOVA test analysis with other independent risk factors revealed no independent correlation of PON1 192R with CVD risk. Polymorphism of PON1 gene inhibits

PON1 activity and activates LDL oxidation, indicating that 192Q is a more effective allele than 192R in preventing LDL oxidation (Raza ST et al, 2022) confirming the earlier observation that R allele carrier has a higher risk of developing CVD than Q allele carriers. According to (Kumar R et al.2021), North Indian population with the PON1 RR genotype have a 9-fold increased chance of developing CAD, and those with the PON1 QR genotype have a 4-fold increased risk. However, in our study sample size is small and a limitation to study the broad spectrum of this study.

Furthermore, a correlation between PON1 polymorphisms and CAD risk was shown in Patients with CAD and hyperlipidemia and had considerably higher frequencies of the 192R allele and 192RR genotype, 192QR + 192RR genotype too exhibited the lower levels of PON1, and the Q192R polymorphism was linked to low serum PON1 concentrations (Chen H, et al. 2018).

PON1 activity was lower in patients with substantial coronary stenosis (SCS) than in controls as shown in a previous study that examined PON1 genotype, activity and lipid profile as well as their associations with SCS in the Tunisian population indicating that low activity of PON1 was linked to the allele 192R.

PON1 192RR genotype is linked to higher risk of SCS in the presence of diabetes (Rejeb J, et al. 2013). However in contrast a study by (Mackness B, et al. 2012) have shown that PON1 activity was favorably correlated with the 192R allele in a study involving 589 patients (419 Caucasian, 120 South Asian, and 50 other) also PON1 concentration was positively correlated with the 192R allele in South Asian population and negatively correlated with Caucasian race and the length of diabetes.

According to (Liu T, et al. 2014) in the Chinese Han population, the 192R allele indicated to be the risk factor of CAD and also reduced PON1 activity and its concentration in CAD patients. According to, (Kaur S, et al.2018) in the Asian Indian community, the Q192R polymorphism in the PON1 gene may be a susceptible gene linked to an elevated risk of CAD.

The contraindications were found in Caucasian population. However, South Asian population showed a strong correlation between 192R allele with CVD in diabetic patients. Same was observed in our study conducted in South Asian Indian population from northern region of India. However, we propose a case control study with larger sample size may be conducted towards a conclusive outcome.

7. Conclusion

According to our findings, the PON1 192R allele is linked to type 2 diabetes (T2DM) and may be involved in the disease's pathophysiology. Q192R may be an emerging risk factor for CVD in patients with type 2 diabetes because it is linked to CVD and decreased serum enzyme concentration of PON1.

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ANNEXURES

PROFORMA

<table>
<tr><td colspan="3">DEMOGRAPHIC DATA</td></tr>
<tr><td>Name</td><td colspan="2">Mr./Mrs./Ms.:-</td></tr>
<tr><td colspan="2">Date of Birth:-</td><td>Sex: Male/Female</td></tr>
<tr><td colspan="2">Weight (in Kg):-</td><td>Height (in cm):-</td></tr>
<tr><td colspan="3">Address:
Street: _____ City: _____
Stare: _____ Pin Code: _____</td></tr>
<tr><td colspan="2">Mobile No.:</td><td>Email:</td></tr>
<tr><td colspan="3">Education Status:</td></tr>
<tr><td colspan="3">Occupation:</td></tr>
<tr><td colspan="3">At which age T2DM diagnosed?</td></tr>
</table>

SANTOSH DEEMED TO BE UNIVERSITY (GHAZIABAD)

INFORMED CONSENT

I……Son/Daughter/Wife of…….resident of…….hereby declare that I give my full, free and voluntary informed consent in the thesis PON1 GENE POLYMORPHISM AND ITS INFLUENCE ON CARDIOVASCULAR THREAT IN TYPE 2 DIABETES MELLITUS. Investigator …….has informed me to my full satisfaction, in the language I understand, about the purpose, nature of study and various laboratory investigations for the study to be carried out. – I have been informed about the duration of the study and instructions to be followed during the study period.

I give full consent for being enrolled in the above study and I reserve my rights to withdraw from this study, whenever I wish without prejudice of my right to undergo further treatment at Santosh Hospital, Ghaziabad U.P

Patient's/ Patient's relative's signature Doctor's signature

Or thumb impression

Name: Date:

We have witnessed that the patient signed the above form in the presence of his free will after fully understanding its content.

Signature of Witness

Name: Signature of Investigator

Name – Apoorva Tiwari

Designation- Student

(Msc Medical Biochemistry, 2021-2024)

Contact No- 9821798602

संतोष डीम्ड यूनिवर्सिटी (गाज़ियाबाद)

सहमति पत्र (सुचित)

मैं.....बेटा/बेटी/ पत्नी.....निवासी.....घोषण करता हूं कि शोधकता .....ने मुझे मेरी भाषा में अपने अध्ययन के उद्देश्य के बारे में और अध्ययन के दौरान जाने अपनायी जाने वाली विभित्र प्रयोगशाला जांच के बारे में सूचित कर दिया है। मुझे अध्ययनकी अवधि और उसके दौरान अपनाए जाने वाले निर्देश भी मेरी भाषा में समझा दिए गए हैं। मैं उपरोक्त अध्ययन में बिना किसी दबाव के दाखिला ले रहा हूँ और पूर्ण सहमति दे रहा हूं। सन्तोष अस्पताल, गाजियाबाद में अपने आगे के उपचार पर बिना प्रतिकूल प्रभाव डाले इस अध्ययन से और प्रयोगशाला जांच की प्रकृति से में जब चाहूँ बाहर जाने के अपने अधिकार को सुरक्षित रख रहा हूं।

रोगी के रिश्तेदार के हस्ताक्षर डॉक्टर के हस्ताक्षर।

नाम

दिनांक:

मेरी उपस्थिति में मरीज ने पूरी तरह से डाकटर के बताए गए अध्ययन और निर्देशों को समझने के बादअपनी स्वतंत्र इच्छा से ऊपर के फार्म पर हस्ताक्षर किए हैं।

हस्ताक्षर

नाम: .....

पदनामः .....

संपर्क नंबर: .....

DETAILS OF PATIENT INFORMATION SHEET

HUMAN RESEARCH PROPOSAL

1. Identifying Data

Name of Researcher: Apoorva Tiwari

Department: Biochemistry

Title of Project: PON1 GENE POLYMORPHISM AND ITS INFLUENCE ON CARDIOVASCULAR THREAT IN TYPE 2 DIABETES MELLITUS

2. Description of Human Subjects Safeguards and Rights:

a) Possible psychological of physical risk of discomfort, involved in research If Yes/No (if no, skip time B)
If yes, answer the following

Why necessary?

1. To have the common disease
2. Specificity and sensitivity of the treatment

b) Make sure the following questions are answered in your research proposal on collection of Data:

1) When will it take place?

2) What length of time needed for complete examination of the patient?

3) What period of the time needed?

4) Which specific place within institution?

5) How much population required including number of subjects?

6) Name of instrument to be used for data collection?

7) Methodology to be used (write details)?

c) Will subjects be given research results if they so request? Yes / No

LETTER OF ETHICAL CLEARANCE FOR DISSERTATION

FROM REGISTRAR OFFICE

SANTOSH
Deemed to be University
(Established u/s 3 of the UGC Act, 1956)

F. No. SU/R/2024/1344[5] Dated: 17.05.2024

Subject: Grant of Ethical Clearance for **Dissertation Protocol** Topic **"Paraoxonase 1 Gene Polymorphism",** submitted by **APOORVA TIWARI,** student of M.Sc. Medical BIOCHEMISTRY, 2021 Batch.

*****

With reference to the request for grant of Ethical Clearance for the Dissertation Protocol entitled **"Paraoxonase 1 Gene Polymorphism", APOORVA TIWARI** is informed that the **Dissertation Protocol** submitted by him/her was considered by the **Screening Committee** of the **Allied Health Sciences** in its meetings held on 07.02.2024 and 29.02.2024. The recommendations of the **Screening Committee** were considered in detail by the **Institutional Ethics Committee** in its meeting held on **15.05.2024** and the same was **approved** by the **Ethics Committee** of the **Santosh Deemed to be University, Ghaziabad, Delhi NCR**.

He /She is informed accordingly for further necessary action.

17/5/24

[Dr. EZHILRAJAN. K]
REGISTRAR

APOORVA TIWARI
M.Sc. Medical BIOCHEMISTRY.

Copy to:

1. The Vice Chancellor
2. The Dean – Academics
3. The Dean, SMC&H
4. The Dean – Research
5. The Director - IQAC
6. HOD/ Course In-charge of the concerned Department.

STATISTICIAN CERTIFICATE

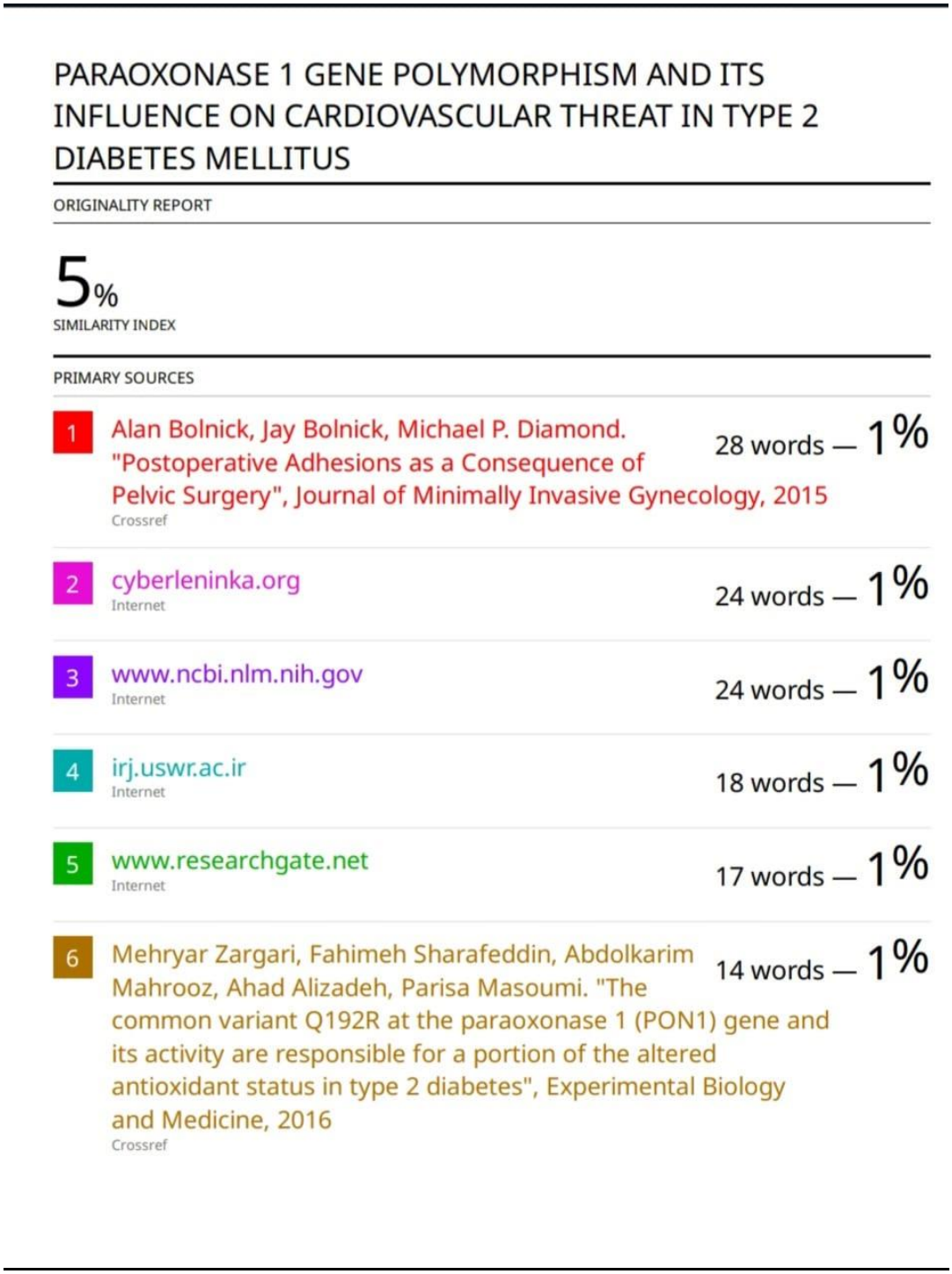
This is to certify that the statistical analysis component of the thesis titled “PON1 GENE POLYMORPHISM AND ITS INFLUENCE ON CARDIOVASCULAR THREAT IN TYPE 2 DIABETES MELLITUS” done by Apoorva Tiwari in the Department of Central Research Facility, Santosh Medical College and Hospital, Ghaziabad has been conducted under my direct guidance and supervision.

All the statistical calculations of this study are authentic and correct to the best of my knowledge.

Wish her all the success in her life.

Dr. Amod Kumar
Assistant Professor cum Statistician
Department Of Community Medicine
Santosh Deemed To be University, Ghaziabad.
Email : Amod.ism01@gmail.com

PLAGIARISM CERTIFICATE



PARAOXONASE 1 GENE POLYMORPHISM AND ITS INFLUENCE ON CARDIOVASCULAR THREAT IN TYPE 2 DIABETES MELLITUS

ORIGINALITY REPORT

5%
SIMILARITY INDEX

PRIMARY SOURCES

1	Alan Bolnick, Jay Bolnick, Michael P. Diamond. "Postoperative Adhesions as a Consequence of Pelvic Surgery", Journal of Minimally Invasive Gynecology, 2015 Crossref	28 words — 1%
2	cyberleninka.org Internet	24 words — 1%
3	www.ncbi.nlm.nih.gov Internet	24 words — 1%
4	irj.uswr.ac.ir Internet	18 words — 1%
5	www.researchgate.net Internet	17 words — 1%
6	Mehryar Zargari, Fahimeh Sharafeddin, Abdolkarim Mahrooz, Ahad Alizadeh, Parisa Masoumi. "The common variant Q192R at the paraoxonase 1 (PON1) gene and its activity are responsible for a portion of the altered antioxidant status in type 2 diabetes", Experimental Biology and Medicine, 2016 Crossref	14 words — 1%

Master chart for demographic parameters of type 2 diabetes mellitus patients

SAMPLE ID	Gender	Age	SBP	DBP	Height (cm)	Weight (kg)	BMI
T2DM001	Female	74	160	80	160	65	25.4
T2DM002	Male	53	150	85	175	80	26.1
T2DM003	Female	45	140	85	155	70	29
T2DM004	Male	94	160	90	170	75	25.9
T2DM005	Male	54	155	80	180	85	26.2
T2DM006	Female	48	145	95	165	70	25.7
T2DM007	Female	66	160	85	160	68	26.6
T2DM008	Female	69	155	80	165	75	27.5
T2DM009	Male	54	165	85	175	90	29.4
T2DM010	Female	42	150	80	160	72	28.1
T2DM011	Male	56	155	85	170	82	28.4
T2DM012	Female	47	160	90	165	80	29.4
T2DM013	Male	48	145	80	180	90	27.8
T2DM014	Female	45	155	85	160	70	27.3
T2DM015	Female	40	150	85	155	65	27.1
T2DM016	Female	50	160	90	160	70	27.3
T2DM017	Female	65	155	85	165	75	27.5
T2DM018	Female	72	150	80	160	70	27.3
T2DM019	Female	70	155	85	160	75	29.3
T2DM020	Female	36	145	80	160	65	25.4
T2DM021	Male	61	155	85	175	85	27.8
T2DM022	Male	62	160	90	170	80	27.7
T2DM023	Female	38	145	85	155	70	29
T2DM024	Male	51	165	85	180	90	27.8
T2DM025	Male	51	170	90	175	95	31
T2DM026	Female	43	150	80	165	70	25.7
T2DM027	Female	66	160	90	165	80	29.4
T2DM028	Male	71	155	85	180	85	26.2
T2DM029	Male	45	150	80	175	80	26.1
T2DM030	Male	45	155	85	175	85	27.8

Master chart for Biochemical parameters and PON1 genotype of type 2 diabetes mellitus patients

Name	HbA1c	Total Cholesterol	TGL	HDL	LDL	VLDL	FBS	PON1 genotype
T2DM001	7.3	160	130	45	93.5	26	150	AA
T2DM002	7.4	149	134.5	36	86.1	26.9	197.5	AA
T2DM003	8.8	157	129.8	41	90.04	25.96	205.86	AG
T2DM004	7.1	224	200	38	154.9	31.1	157.07	GG
T2DM005	7.6	156	148.9	25	79	30.5	193.9	GG
T2DM006	11.3	208	180	42.5	38	36	220	GG
T2DM007	7.6	165	150	35	89.22	30	170	AA
T2DM008	7.6	120	110	44.8	49.18	22	142.5	GG
T2DM009	9.5	122	160.5	38	59.1	32.1	185.6	AG
T2DM010	9.3	166	138	42.4	96	27.6	160.6	GG
T2DM011	8.3	175	123.6	36	114.28	24.72	147.6	AG
T2DM012	11.3	145	247.7	44.8	50.66	49.54	217.6	GG
T2DM013	6.5	180	174.6	38	107.08	34.92	107.6	GG
T2DM014	6.8	160	130.9	38	95.82	26.18	160.2	AA
T2DM015	6	148	129.2	38	84.16	25.84	132.7	AG
T2DM016	6.5	175	105.4	37	116.92	21.08	91.7	GG
T2DM017	8.3	210	140.3	46.6	135.34	28.06	136.1	AA
T2DM018	8.2	144	68.2	43	87.36	13.64	126.4	AG
T2DM019	8.2	177	97.5	38	119.5	19.5	127.5	AG
T2DM020	6.5	116	99.6	36	60.08	19.92	105.8	GG
T2DM021	7.7	142	130.5	36	79.9	26.1	143.1	AA
T2DM022	6.5	115	96.4	38	57.72	19.28	201.3	GG
T2DM023	9.1	155	173.8	38	82.24	34.76	167.1	AA
T2DM024	8.5	282	302.1	39.5	182.02	60.42	223.1	AA
T2DM025	15.1	199	159.6	51.4	115.68	31.92	380.4	GG
T2DM026	6.5	186	141.9	41.6	116.02	28.38	123.9	AA
T2DM027	10.7	90	115.1	38	28.98	23.02	221.1	GG
T2DM028	7	186	155.4	41	113.92	31.08	137.9	AG
T2DM029	9	127	123.2	36	66.36	24.64	161.5	GG
T2DM030	7.3	149	67.4	41	94.52	13.48	127.7	GG

Master chart for demographic parameters of healthy controls

SAMPLE ID	Gender	Age	Height (cm)	Weight (kg)	SBP	DBP	BMI
C001	Male	18	175	65	120	85	21.22
C002	Male	23	180	75	125	85	23.15
C003	Female	40	165	68	130	85	24.98
C004	Male	55	170	85	140	90	29.41
C 005	Male	37	175	80	135	88	26.12
C006	Female	40	160	62	120	80	24.22
C007	Female	37	170	70	125	80	24.22
C008	Female	46	155	55	110	75	22.88
C009	Female	34	165	60	125	80	22.04
C010	Female	42	160	75	130	85	29.3
C011	Male	35	172	78	128	82	26.43
C012	Male	37	175	85	130	85	27.76
C013	Male	49	165	70	120	80	25.71
C014	Male	23	180	72	125	80	22.22
C015	Male	37	180	74	120	80	22.84
C016	Female	58	158	68	130	85	27.16
C017	Female	29	165	60	120	80	22.04
C018	Male	27	175	85	125	80	27.76
C019	Male	58	170	75	120	80	25.95
C020	Male	61	160	70	125	85	27.34
C021	Male	32	180	85	130	85	26.23
C022	Male	66	170	80	135	90	27.68
C023	Male	47	175	85	130	85	27.76
C024	Female	33	160	62	120	80	24.22
C025	Female	49	165	70	125	80	25.71
C026	Female	40	160	65	120	80	25.39
C027	Female	36	165	70	130	85	25.71
C028	Female	40	160	63	120	80	24.22
C029	Female	26	165	68	125	80	24.98
C030	Female	69	155	60	130	85	24.96

Master chart for Biochemical parameters and PON1 genotype of healthy controls

SAMPLE ID	HbA1c	Total Cholesterol	TGL	HDL	LDL	VLDL	FBS	PON1 genotype
C001	5.4	138	58.6	47.2	79	11.72	74.8	AG
C002	5.3	105	52.8	36	58	10.56	94.2	AA
C003	5.5	160	63	42	105	12.6	100.4	AA
C004	5.6	247	116.7	41.1	182	23.34	88.8	AG
C 005	5.3	158	181.4	38	83.72	36.28	109.7	AA
C006	5.6	145	121.3	41	79.74	24.26	91.9	AG
C007	5.4	161	112	41	97.6	22.4	126	AA
C008	5	100.2	45	37	75	9	96.8	AG
C009	5.2	87.2	136	99.4	42	19.88	96.8	GG
C010	5.4	197	134.5	36	134.1	26.9	84.3	AA
C011	5.3	169	130.4	38	104.92	26.08	106.2	GG
C012	5.5	175	253.1	41	83.38	50.62	115.1	AA
C013	5.2	111	86.7	41	52.66	17.34	94.2	AG
C014	5.1	172	86.1	38	116.78	17.22	98.1	AA
C015	5.2	172	79.7	42.9	113.16	15.94	96.6	AG
C016	5.2	188	99.4	44.7	123.42	19.88	104.8	AG
C017	5.5	121	60.2	38	70.96	12.04	90.2	GG
C018	5.4	215	213.7	37	135.26	42.74	90.5	AG
C019	5.5	221	81.3	63.6	141.14	16.26	89.2	GG
C020	5.2	147	93.9	28.8	99.42	18.78	95.3	AA
C021	5.5	213	188.9	40	135.22	37.78	99.3	AG
C022	5	185	141.2	41	115.76	28.24	114.9	AA
C023	5.3	186	171.1	41	110.78	34.22	98.5	AA
C024	5.4	165	83.1	36	112.38	16.62	102.9	AA
C025	5.5	177	95	38	120	19	100.7	AA
C026	5.3	141	75.7	38	87.86	15.14	87.3	AG
C027	5.1	186	129	43.3	116.9	25.8	85.7	GG
C028	5.3	128	104.6	38	69.08	20.92	96.8	AA
C029	5	139	142.6	39	71.48	28.52	98.3	AA
C030	5.5	176	76.9	51.8	108.82	15.38	103.4	AA